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Metal Particles Are Inappropriate for Testing a Postulate of Extrapulmonary Transport

Exposure to ambient air pollution particles has been associated with increased human morbidity and mortality, much of which is nonpulmonary. One proposed explanation of this extrapulmonary tissue injury is a transport of the particles outside of the respiratory tract. In the August 2006 issue of *EHP*, Elder et al. (2006) tested a postulate of extrapulmonary transport of particulate matter (PM). Specifically, the authors focused on the potential translocation of particles by olfactory neuronal pathways to the central nervous system. Comparable to previous studies on systemic transport of PM, they used a metal particle (i.e., a manganese oxide). Elder et al. measured tissue Mn concentrations in an effort to establish transport of the particle.

Past research has repeatedly demonstrated that components of PM can be solubilized, mobilized, and transported to tissues outside the respiratory tract independently of the original particle (e.g., nicotine transport from the cigarette-smoke particle to the blood and central nervous system of the smoker). Metals are among those components that can be solubilized and mobilized from particles and distributed systemically without the translocation of the particle from the original site of deposition in the respiratory tract. To meet the demands of growth and homeostasis, living systems frequently acquire metals from particles using some combination of chemical reduction and direct chelation (Currie and Briat 2003). Elder et al. (2006) reported that the transport of Mn, with elevated concentrations of the metal in extrapulmonary tissues, supports a translocation of the original PM into the central nervous system. However, their use of Mn oxide to test any postulate of extrapulmonary transport of a particle is inappropriate as a result of the *in vivo* availability of both reductants (e.g., superoxide, ascorbate, glutathione) and chelators (e.g., transferrin, lactoferrin, citrate, urate) in the mammalian respiratory tract. These reductants were not available in their *in vitro* solubility tests (performed in normal saline), the results of which they used to support their conclusion of direct *in vivo* translocation of particles. Elevated concentrations of the metal in extrapulmonary tissues do not prove direct translocation of the original

PM, but rather reflect solubilization and mobilization of the Mn from the oxide particle, with subsequent distribution. Additionally, the rapidity of change in metal concentration at an extrapulmonary site should not support a direct translocation because the time required for solubilization, mobilization, and systemic transport of a metal from any particle has never been defined; the required time for such transport is predicted to be short for ultrafine particles, which have an increased surface area available for rapid interactions of the PM with endogenous reductants and chelators. What is required to prove the existence of an extrapulmonary transport of PM is employment of a particle with components that cannot be independently solubilized, mobilized, and transported from the site of its original deposition. A carbon-based PM would be appropriate.

When evaluating previous investigations for evidence of extrapulmonary transport of carbon-based particles, there is little to support translocation of such PM outside the respiratory tract. A recent study by Mills et al. (2006) showed no translocation of insoluble, radiolabeled ultrafine carbon particles from the lung into the bloodstream of humans. The small amount of radioactivity they found in the bloodstream and other organs within 6 hr of inhalation could be attributed entirely to the small amount of leached, soluble radiolabel from the particles. Among miners who were exposed to coal dust at 1,000 times the concentrations of ambient air pollution PM, LeFevre et al. (1982) observed the particle in only the lung and the reticuloendothelial system; the presence of the particle in the reticuloendothelial system reflects the transport of PM after sequestration within phagocytes, because the particles are always detected within these cells at these sites.

Finally, decades of research have provided no evidence of an extrapulmonary transport (including via olfactory neuronal pathways) of particles associated with cigarette smoking; smoking exposes the individual to literally kilograms of a particulate combustion product that includes ultrafine particles. While again demonstrating that a metal component of a particle can be solubilized, mobilized, and transported from the site of original deposition, Elder et al. (2006) provided no evidence to support a disbursement of the actual PM to tissues outside the respiratory tract.

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Metal Particles and Extrapulmonary Transport: Oberdörster and Elder Respond

We appreciate the opportunity to address the points raised by Ghio and Bennett in their letter. The pH of our cell-free dissolution studies (Elder et al. 2006) was appropriate because the nasal cavity surface is neutral and does not have airway macrophages and the phagolysosomal pH of nasal epithelial cells is neutral (Johnson 1994). Acidic pH, as in the phagolysosome of alveolar macrophages, dissolves manganese oxide, resulting in increased levels of blood-borne non-particulate Mn. Our neutral pH buffer dissolved only 1.5% of Mn oxide nanoparticles in 24 hr. The dissolution buffer was not “normal saline,” as stated by Ghio and Bennett; it was physiologic saline, a “simulated lung fluid” used for decades in studies of man-made fiber dissolution (Potter and Mattson 1991). It was an oversight on our part to omit the exact composition from our article (Elder et al. 2006), which includes citrate (model organic acid) and glycine (model protein component). Citrate is a stronger metal chelator than glycine.

If only soluble Mn translocates, the time required for solubilization would significantly retard the increase of Mn in the olfactory bulb of administered Mn oxide nanoparticles. Instead, we found no difference between the two forms of Mn when we directly compared the translocation rate of the Mn oxide nanoparticles to soluble Mn chloride (Elder et al. 2006), indicating a direct uptake of the Mn oxide nanoparticles by olfactory neuronal structures

and subsequent translocation. It is conceivable that subsequent dissolution in the olfactory system occurs. The rapidity of solid nanoparticle transport along neuronal axons is, indeed, remarkable (~ 2.5 mm/hr), as demonstrated earlier by the arrival in the olfactory bulb of 50 nm gold particles within 30 min after intranasal instillation. This and other studies with gold nanoparticles using transmission electron microscopy detection (reviewed by Oberdörster et al. 2005) demonstrate unequivocally that some metal particles are indeed appropriate for demonstrating solid particle transport across epithelial barriers, refuting the absolute statement in the title of Ghio and Bennett's letter.

Ghio and Bennett suggest that a carbon-based particle would be appropriate for studying ultrafine particle transport and translocation. This is true for elemental and organic carbon only if insoluble *in vivo*. Indeed, study with inhaled ultrafine elemental carbon particles (^{13}C) confirmed their translocation to the olfactory bulb of rats (Oberdörster et al. 2004). In contrast, labeled elemental carbon is problematic, as pointed out by Ghio and Bennett regarding Technegas ($^{99\text{m}}\text{Tc}$ labeled-ultrafine carbon). Recent studies using inhaled Technegas (Mills et al. 2006; Wiebert et al. 2006) showed no translocation in humans, contradicting earlier work (Nemmar et al. 2002). Both leaching of the soluble radiolabel and the inability of the γ -camera to detect small amounts ($\leq 1\%$) of the deposited dose in extrapulmonary organs are significant limitations with this noninvasive technique, resulting in misinterpretations suggesting either significant particle translocation or lack thereof.

Ghio and Bennett cite LeFevre et al. (1982), apparently as evidence that inhaled carbon-based particles do not undergo extrapulmonary transport. However, LeFevre et al. interpreted their findings of high black pigment scores in liver and spleen of coal miners differently—namely, as migration of coal dust in the pneumoconiotic lung into pulmonary lymphatics and then to the systemic circulation, and also as migration of coal mine dust-laden macrophages through the walls of pulmonary blood vessels. Whatever the mechanism, their findings clearly indicate extrapulmonary transport. Heavy silica inhalation exposure has also been found to result in particle accumulation in liver and other extrapulmonary tissues in humans and nonhuman primates (Carmichael et al. 1980; Rosenbruch 1990; Slavin et al. 1985). Ghio and Bennett further state that

Decades of research have provided no evidence of an extrapulmonary transport (including via olfactory neuronal pathways) of particles associated with cigarette smoking.

How many investigators have tried to find cigarette smoke particles in extrapulmonary tissues? We are aware of only one study in rats in which a 25-min inhalation exposure to ^{14}C cigarette smoke resulted in 0.24–0.83% of retained ^{14}C in the liver within 15 min after the short-term exposure (Chen et al. 1989). Does this indicate extrapulmonary particle transport? Possibly yes.

We conclude that the physicochemical characteristics of a nanoparticle—whether metal or not—and the physiologic milieu at the site of deposition in the respiratory tract determine whether extrapulmonary translocation occurs as particle or as solute. A prerequisite for noninvasively measuring this is that the detection method has sufficient sensitivity for identifying the analyte at expected low translocation rates.

Regarding our nasal translocation study in rats (Elder et al. 2006), we conclude that inhaled Mn oxide nanoparticles are taken up by the nasal olfactory neuronal pathway as solid particles rather than being slowly dissolved first at neutral pH. For the alveolar region where dissolution is expected to be more rapid, this does not apply.

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Limitations of WTC Five-Year Assessment

We have learned much about the respiratory disorders since the exposures of responders at the World Trade Center (WTC) site, especially from the publications of Prezant and colleagues about the presentations, follow-up, and impairments of pulmonary function and bronchial reactivity of the fire fighters and emergency medical technicians of the New York City Fire Department (Banauch et al. 2003, 2005, 2006; Prezant et al. 2002). These reports are especially informative because of the availability of preexposure clinical and spirometric data.

We appreciate the report of much-awaited results among 9,442 workers from the WTC Worker and Volunteer Medical Screening Program (Herbert et al. 2006). Because of the potential for major illness, the large number of subjects at risk, and the resultant enormous public interest, it is important that the information reported be properly understood. A number of limitations in this report must be pointed out.

Although the title identified this report (Herbert et al. 2006) as a 5-year assessment, screening examinations were performed between 16 July 2002 and 16 April 2004, < 1 year through < 3 years after 11 September 2001. There were no follow-up examinations, either at the 5-year or at any other interval.

Summary conclusions (Herbert et al. 2006), heavily reported in the media, lump all respiratory symptoms:

... 69% reported new or worsened respiratory symptoms while performing WTC work. Symptoms persisted to the time of examination in 59% of these workers.

The 69% with “any respiratory symptom” included 23.3% with no “lower respiratory symptoms.” A far smaller percentage of all workers (17.3%) complained of what may be considered the most important respiratory symptom, dyspnea, which was not quantified by any standard scale. Such a reliance on symptoms is subject to recall biases both for symptoms present before 9/11 and for the onset, worsening, and persistence of symptoms after 9/11.

Because physical examination and chest radiographs were unrevealing (Herbert et al. 2006), the only objective results were from

pulmonary function tests. These were confined to spirometry, which does not provide insight into all aspects of respiratory impairment. The data presented by Herbert et al. (2006) are limited. Mean values for subsets (classified by WTC exposure, previous smoking history, etc.) are not given. Despite the frequency of cough (42.8%), wheeze (15.1%), and chest tightness (15.4%) and the common diagnoses of asthma/reactive airways dysfunction, only 7.6% of all responders showed airway obstruction, defined as a ratio of forced expiratory volume in 1 sec (FEV₁) to forced vital capacity (FVC) less than the 5th percentile of the reference population. Unlike virtually all spirometric surveys of a large population (reviewed by Miller et al. 1991), Herbert et al. (2006) found little difference in impairment by smoking status. Most spirometric impairments were classified as restrictive, uncharacteristic of the symptoms and clinical diagnoses. This frequency of low FVC (22.7%) raises several issues: *a*) the effects of other clinical factors not reported on, such as obesity; *b*) technical considerations in subject performance or technician monitoring of the FVC maneuver, despite the investigators' attention to these; and *c*) the appropriateness of the reference-predicted values.

We await further information and follow-up from these investigators, including results of additional diagnostic procedures not included in routine screening. These include a wider array of pulmonary function tests (full lung volumes, diffusing capacity), measurement of bronchial reactivity, computed tomography scans, and—in appropriate patients—bronchoalveolar lavage and lung biopsies, which would truly elucidate the respiratory disorders following WTC exposure.

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WTC Five-Year Assessment: Herbert et al. Respond

In our article (Herbert et al. 2006), we described the establishment of the World Trade Center (WTC) Worker and Volunteer Medical Screening Program and presented results of screening examinations undertaken between 2002 and 2004 among a heterogeneous group of 9,442 WTC responders.

Miller expresses concern about the validity of self-reported upper and lower respiratory symptoms in WTC responders. He notes correctly that self-reported symptoms are inherently subjective. However, symptoms cannot merely be dismissed as unimportant, especially when they are persistent and when, as was the case here, the pattern of their occurrence closely parallels severity of exposure. We reported that symptoms were most common among those responders who arrived earliest at the WTC site and who consequently suffered the heaviest exposures to the highest levels of dust and smoke (Herbert et al. 2006). This finding has high inherent biological plausibility. To be sure, the potential for recall bias is always present in a symptom-based survey. In reality, however, recall bias could be of concern only if we had reason to believe that responders in different exposure groups recalled past and current symptomatology differently. Finally, to further ensure the validity of our findings, we buttressed our assessment of symptoms with chest X rays and pulmonary function tests.

Miller also expresses concern that objective results were “confined to spirometry, which does not provide insight into all aspects of respiratory impairment.” Although we recognize the limitations of spirometry, a large-scale screening program has practical restrictions in testing that can be accomplished. In fact, in Miller's own 1991 survey of a population 10 times smaller than our own (Miller et al. 1991), only spirometry was used as a screening tool. Miller observes that our results were “unlike virtually all spirometric surveys of a large population” since there was “little difference in impairment by smoking status.” We would agree with Miller that our population was distinct by the very nature of the exposures

involved and that this should be considered in evaluating the lack of difference in impairment based on smoking status. One speculation is that the overwhelming exposure to toxic chemicals at the WTC disaster may have masked differences between smokers and nonsmokers.

Miller erroneously states that most spirometric impairments were classified as “restrictive.” We were quite careful not to use this term because it cannot be confirmed by spirometry alone. Instead we chose the designation of low forced vital capacity (FVC) (Herbert et al. 2006). Like Miller, we were surprised by this finding as well as by the observation that fewer responders had reversible airway obstruction, which would have confirmed asthma in those with asthma-like symptoms. However, asthma is by its very nature intermittent, and spirometry tests are only a “snapshot in time,” so normal spirometry results do not rule out asthma. Unfortunately, we were unable to provide inhalation challenge tests for the cohort because of the constraints of conducting a large multicenter clinical screening program.

We listed the many possible reasons for a high prevalence of a low FVC in the “Discussion” of our article (Herbert et al. 2006). One member of our working group (G.S.) is currently leading an initiative to estimate the individual contribution of each of these factors by describing the results of additional diagnostic procedures not included in routine screening.

Examinations of the WTC population continue and are expected to proceed for many years to come. As of 31 December 2006, we have examined > 18,500 WTC responders and provided follow-up examinations to > 7,000. We expect to report on findings from those examinations within the next year. In addition, we will be reporting further on the relationship between symptoms and screening spirometry. These analyses should provide further insight into the potential pulmonary impairment of individuals exposed at the WTC disaster.

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ERRATUM

In Table 2 of the the commentary by Marsee et al. [*Environ Health Perspect* 114:805–809 (2006)], the value for the 75th percentile of monobenzyl phthalate (MBzP) was incorrect; the correct value is 0.92.

The authors regret the error.